Silica nanoparticles: synthesis and functionalization for drug delivery application

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CERTIFICATE OF STUDY

National Institute of Technology
Rourkela

This is certify that **Mr. Animesh Mondal** and **Ms.Smruti Mishra**, students of M.Sc. in Chemistry (2012-2014), in NIT Rourkela, Odisha, have carried out their dissertation work on "***Silica nanoparticles: synthesis and functionalization for drug delivery application***" under my supervision and guidance as a partial fulfillment of the degree of M.Sc. in Chemistry. The thesis embodies original work done by them and deserves merit for consideration for the degree. No results or any part of the result have been submitted anywhere for degree or equivalent qualification.

Date: Supratim Giri

Place: NIT Rourkela (supervisor)

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Animesh Mondal

Smruti Mishra

INTRODUCTION

The research involving mesoporous silica is continuing to expand since their discovery by the Mobil research scientists about a couple of decades ago [1]. The ease of producing porous, monodispersed silica materials with particle size dimension ranging in few hundred nanometers have attracted a wide range of applications [2]. As these silica nanoparticles can hold a relatively large amount of host cargo compared to their volume, there particles act as nanocarriers and often find interesting applications ranging from drug delivery to bio-sensing [3]. Mesoporous silica nanomaterials are typically synthesized from surfactant molecules acting as a templates or structure driving assembly. The pore diameters, orderedness of pores, pore wall thickness *etc.* can be easily controlled by choosing a particular type of surfactant molecule and varying the reaction conditions [4]. Such chemical control provides a pathway to generate engineered mesoporous silica materials that can have a very high surface area up to 1000 m^2/g and the pore diameters can also be varied from 2 to 10 nm. As a result, these porous materials can act excellent host for a wide range of biomolecules. Further chemical functionalization of the pore wall surface of the silica is also possible so that using non-covalent interactions *e.g.* electrostatic, hydrogen bonding and van der Waal interaction, molecular cargos can be stored inside the porous channels of these materials. For such flexibility of making tailored porous silica, these materials exhibit much higher loading of host molecules compared to that possible using liposome or other polymer conjugates.

In this project, we intended to develop a silica nanoparticle based drug delivery system, which is designed to work for colon cancer model. Colorectal cancer or colon cancer is one of the major causes of human mortality in the world [5]. In every year, several hundred thousands of patients are diagnosed with colon cancer throughout the world. Most of these patients undergo chemotherapy as treatment. However, chemotherapy itself has harmful side effects on human body. In this work we have proposed a silica nanoparticle based model that is designed to deliver drug molecules selectively in the cancer tissues of the colorectal region. We have synthesized silica nanoparticles *via* a surfactant template method. These silica nanoparticles are first loaded with an anticancer drug and then capped with guar gum polymer. Guar gum is a natural non-ionic polysaccharide obtained from a type of leguminous plant *Cyamopsis tetragonoloba,* which is predominantly grown in India [6]. The structure of the guar gum polymer is shown below in Figure 1.

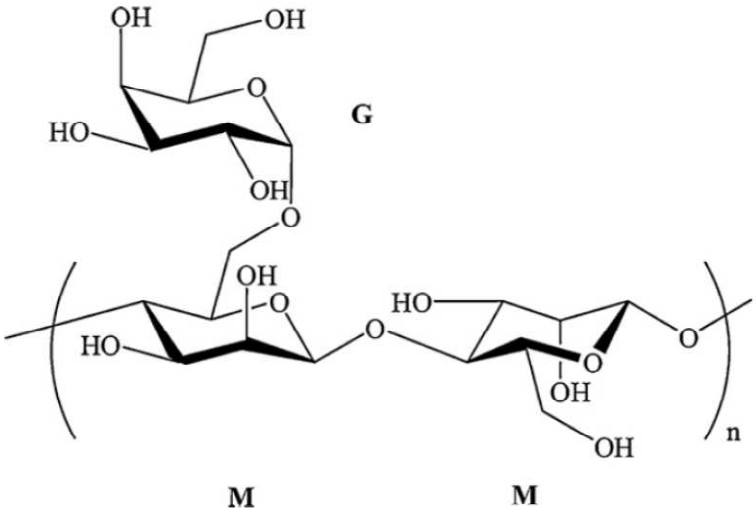


Figure 1. Structure of Guar gum (carbohydrate)

Guar gum consists of a linear backbone of β-1,4-linked D mannose units (M) and a randomly linked α-1,6-linked galactose units (G) as side chains. The side chain imparts solubility in aqueous medium and thus the polymer forms a viscous solution in water.

According to the literature reports, there is a family of glycosidase enzymes (*e.g.* β-D-glucosidase, β-D-galactosidase *etc.*) secreted by bacteria residing in the colonic region [5]. These enzymes are capable of degrading carbohydrate based polysaccharides like guar gum. We hypothesize that when guar gum capped silica nanoparticles (preloaded with drugs) are applied to the colon cancer tissues; these readily available glycosidase enzymes would degrade the guar gum capping. Subsequently, the drug molecules from silica nanoparticles would be released and would target the colon cancer cells. Since the enzyme is present only in the colonic region, the release of the anticancer drug would be site specific. We also plan to use this silica nanoparticle based drug carriers as oral drug delivery system. Once ingested, the particles would reach to the colons and other tissues of the gastrointestinal system but the drugs will only be delivered in the colonic region to target the colon cancer tissues. The design of the silica nanoparticle based drug delivery system is presented in the schematic below.

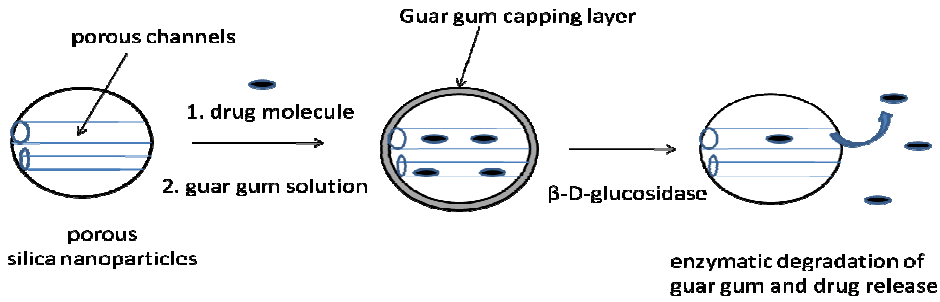


Figure 2. Shematic of guar gum capped silica nanoparticle based drug delivery system

EXPERIMENTAL

Part A: synthesis of silica nanoparticles

Materials: n-cetyltrimethylammonium bromide (CTAB), tetraethylorthosilicate (TEOS), sodium hydroxide (NaOH), hydrochloric acid (HCl), methanol and ethanol were obtained commercially from Aldrich and used as received. De-ionized (DI) water was obtained from Millipore ® standard de-ionizer equipment.

Synthesis: A mixture of CTAB (250 mg, 0.69 mmol), NaOH (2M 875 µl) and DI water (120.0 mL) were heated at 80^{o} C in an oil bath with constant stirring to get a clear solution. To this clear solution, TEOS (1.25 ml, 5.59 mmol) was added with the help of a syringe at a flow rate of 1.25 mL/min. A white precipitate was observed after 7 min 30 seconds from the start of the addition. The reaction mixture was stirred at 80^{o} C for 2 h. The product was isolated by a hot filtration, washed with water first and then ethanol three times each and dried under vacuum. The yield of the product was 458.6 mg. The product was then subjected to surfactant wash. In a typical washing method, the product (458.6 mg) was refluxed in methanol (60.0 mL) with hydrochloric acid (12 N, 2.3 mL) at 65^{o} C for 24 h. The resulting surfactant washed solid product was filtered off and washed with water and methanol several times before drying it under vacuum. The yield of the final washed product was 228.8 mg.

Control experiment: NaOH (2M 875 µl) and DI water (120.0 mL) were heated at 80^{o} C in an oil bath with constant stirring to get a clear solution. To this clear solution, TEOS (1.25 ml, 5.59 mmol) was added with the help of a syringe at a flow rate of 1.25

mL/min. The reaction mixture was stirred at 80^{o} C for 2 h. At the end CTAB was added because no precipitate was obtained. The product was isolated by a hot filtration, washed with water first and then ethanol three times each and dried under vacuum.

Part B: synthesis of functionalized silica nanoparticles prepared by co-condensation method

Materials: n-cetyltriethylammonium bromide (CTAB), tetraethylorthosilicate (TEOS), 3-aminopropyltriethoxysilane (3-APTES) and 3-marcaptopropyltriethoxysilane (3-MPTES), sodium hydroxide (NaOH), hydrochloric acid (HCl), methanol and ethanol were obtained commercially from Aldrich and used as received. De-ionized (DI) water was obtained from Millipore ® standard de-ionizer equipment.

a) Synthesis of Primary amine functionalized silica nanoparticles: A mixture of CTAB (0.25 g, 0.69 mmol), 2M aq. NaOH (0.875 mL) and DI water (120.0 mL) were heated at 80^{o} C in an oil bath with constant and vigorous stirring to get a clear solution. To this clear solution, TEOS (1.25 mL, 5.59 mmol) and 3-APTMS (0.25 mL, 1.03 mmol) were added simultaneously by syringes. The entire addition process was carried out for 1.0 min. A white precipitate was observed after 2 min 4 seconds from the start of the addition. The reaction mixture was stirred vigorously at 80^{o} C for 2 h. The product was isolated by a hot filtration, washed with water first and then ethanol three times each and dried under vacuum. The yield of the product was 564.1 mg. The product was then subjected to surfactant wash. The product (564.1 mg) was refluxed in methanol (60.0 mL) with hydrochloric acid (12 N, 2.3 mL) at 65^{o} C for 6 h. The resulting surfactant washed solid product was filtered off and washed with water and methanol several times

before drying it under vacuum. The yield of the final washed product was 310.2 mg and the sample was designated as PNH_2MSN.

b) Synthesis of thiol-functionalized silica nanoparticles: A mixture of CTAB (0.25 g, 0.69 mmol), 2M aq. NaOH (0.875 mL) and DI water (120.0 mL) were heated at 80^o C in an oil bath with constant and vigorous stirring to get a clear solution. To this clear solution, TEOS (1.25 mL, 5.59 mmol) and 3-MPTES (0.25 mL, 1.03 mmol) were added simultaneously by syringes. The entire addition process was carried out for 1.0 min. A white precipitate was observed after 2 min 4 seconds from the start of the addition. The reaction mixture was stirred vigorously at 80^o C for 2 h. The product was isolated by a hot filtration, washed with water first and then ethanol three times each and dried under vacuum. The yield of the product was 554.1 mg. The product was then subjected to surfactant wash. The product (554.1m g) was refluxed in methanol (60.0 mL) with hydrochloric acid (12N, 2.3 mL) at 65^o C for 6 h. The resulting surfactant washed solid product was filtered off and washed with water and methanol several times before drying it under vacuum. The yield of the final washed product was 329.5 mg and the sample was designated as PSHMSN.

Part C: study of drug loading and release using silica nanopartilces (MSNP)

Materials: 5-Fluorouracil (5-FU) drug was commercially obtained from Sigma Aldrich. Phosphate buffer saline (PBS) of 1 mM ionic strength at pH 7.4 was prepared in distilled water (100 mL) with following composition:

Sodium chloride (0.8 g), Potassium chloride (0.02 g), disodium hydrogen phosphate (0.144 g) and potassium dihydrogen phosphate (0.024 g)

Carbonate bicarbonate buffer solution (CBCS) of pH 10.2 was prepared in distilled water (100 mL) with following composition:

Anhydrous Na_2CO_3 (1.06g, 0.2 M) dissolved in 50 mL distilled water and $NaHCO_3$ (0.84 g) dissolved in 50 mL distilled water. From these solutions, Na_2CO_3 (27.5 mL), $NaHCO_3$ (22.5 mL) was added and made upto 100 mL with distilled water which corresponds to 0.2 M sodium carbonate and bicarbonate buffer.

The following steps were followed to study the drug release from capped silica nanoparticles:

a) Concentration versus absorbance standard plot of 5-FU.
b) Loading of 5-FU in silica nanoparticles.
c) Capping silica with Guar gum

a) Concentration versus absorbance standard plot of 5-FU: 10 mg of 5-FU was dissolved in 1 mL of PBS, which will be considered as a stock solution. To prepare 100 µg/mL solutions, 100 µL from the stock solution was taken in a centrifuge tube and was diluted by PBS using micropipette.

From this 100 µg/mL solution, concentration from 5 µg/mL to 90 µg/mL solutions (each 3 mL) can be prepared by serial dilution.

Table for solutions of different concentration:

S.No.	Concentration of Solution (in µg/mL)	Volume of solution (100 µg/mL) in µL	UV-Absorbance (266nm)
1	5	150	0.188
2	10	300	0.375
3	20	600	0.751
4	30	900	1.132
5	40	1200	1.540
6	50	1500	1.713
7	60	1800	2.234
8	70	2100	2.600
9	80	2400	2.803
10	90	2700	3.210

From 10 mg/mL of 5-FU solution, the concentration of 120 µg/mL to 150 µg/mL solutions (3 mL each) was prepared.

Table for solutions of different concentration:

S.No.	Concentration of Solution (in µg/ml)	Volume of Solution (10 mg/mL) in µL	UV-Absorbance
1	120	36	3.545
2	130	39	3.499
3	150	45	3.569

b) Loading of 5-Fu in silica nanoparticles: PBS (2700 µL), 5-FU solution (300 µL) and sub-100 MSN (30 mg) were taken in a 10 mL beaker and stirred for 24 hours. Product was centrifuged at 5000 rpm for 20 minutes. The supernatant liquid was isolated in a centrifuge tube to take the UV-absorbance value and again it was transferred to the residue.

c) Capping silica with Guar gum: PBS (10 mL), CBCS (10 mL) and 0.1 M HCl (10 mL) taken in three 50 mL conical flask respectively. To each conical flask 0.1% Guar gum was added and dissolved by stirring. After dissolution, 3 mL of them was taken in three 10 mL beaker respectively and 15 mg of sub-100 MSN was added to each beaker, stirred for 24 hours. Centrifuged (at 13,000rpm) and the residues obtained were lyophilized. FT-IR data was recorded for each sample to analyze for capping of silica nanoparticles (fig. 16, 17, 18).

d) Capping silica nanoparticles containing 5-FU with Guar gum:

3 mL of 80ug/mL concentration of 5-FU was taken in 10 mL beaker. To this was added 30 mg of silica nanoparticles, the mixture was stirred for 24 hours at room temperature. Subsequently, 0.1% Guar gum was added and dissolved by stirring for overnight. The mixture was then centrifuged at 13,000 rpm; supernatant was collected to analyze for absorbance (at 266nm).The blank was set against 0.1% Guar gum and 80 µg/mL of 5-FU.

RESULTS AND DISCUSSION

Characterization analysis: Scanning electron microscopy (SEM) was conducted in FEI-FESEM (Nova nano SEM) operated at 5 kV with gold coating for 2 min. Fourier transform infrared analysis (FTIR) was done using a Perkin-Elmer FTIR Spectrometer. UV-vis absorbance was measured using Shimadzu, UV-2450 Spectrophotometer.

Part A: characterization of silica nanoparticles

The silica nanoparticles were prepared from cetyltrimethylammonium bromide (CTAB) based surfactant templates, following a literature reported procedure [7]. After the synthesis, the CTAB molecules were removed from the silica structures by washing the silica materials in acidic solution. The surfactant washed silica materials were analyzed by field emission scanning micrograph (FESEM) after a standard gold coating on the silica surface. The SEM image is shown in Figure 2. The higher magnification image of the SEM study is also shown in Figure 3. From the SEM results it is evident that the silica materials are formed as spherical particles with an average diameter of around 100 nm.

To characterize the elements of the materials, energy dispersive X-ray spectroscopy (EDS) analysis was carried out and the results are shown in Figure 5. Apart from the Au peaks originating due to gold coating, characteristic X-ray peaks from elemental Si and O are the only two other peaks. This result conclusively proves that the resulting nanoparticles are made of silica.

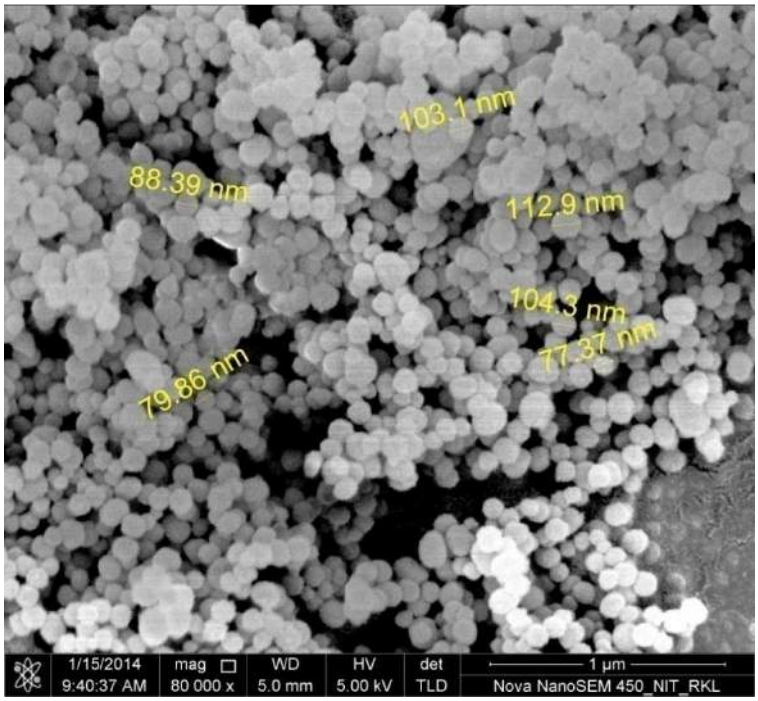


Figure 3. FESEM image of silica nanoparticles obtained from CTAB templates.

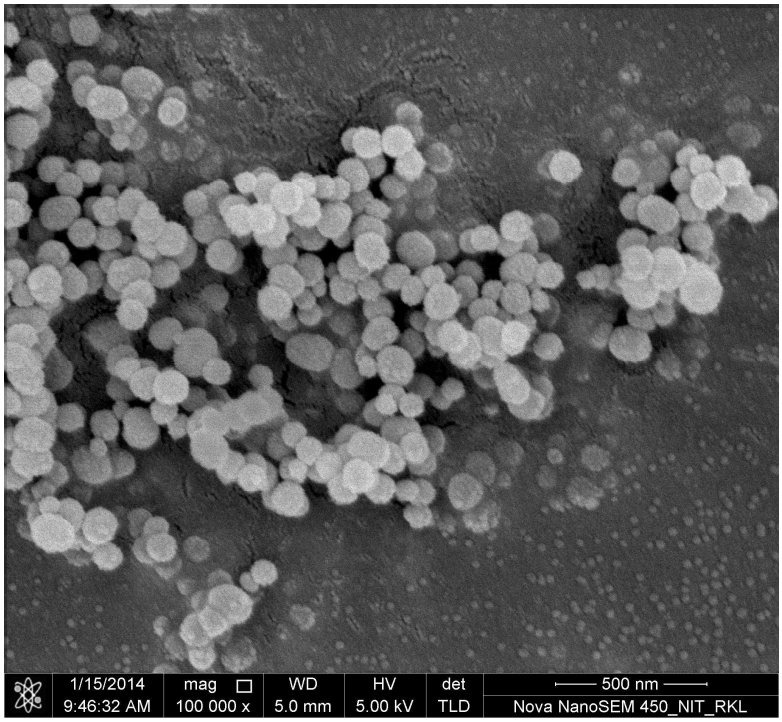
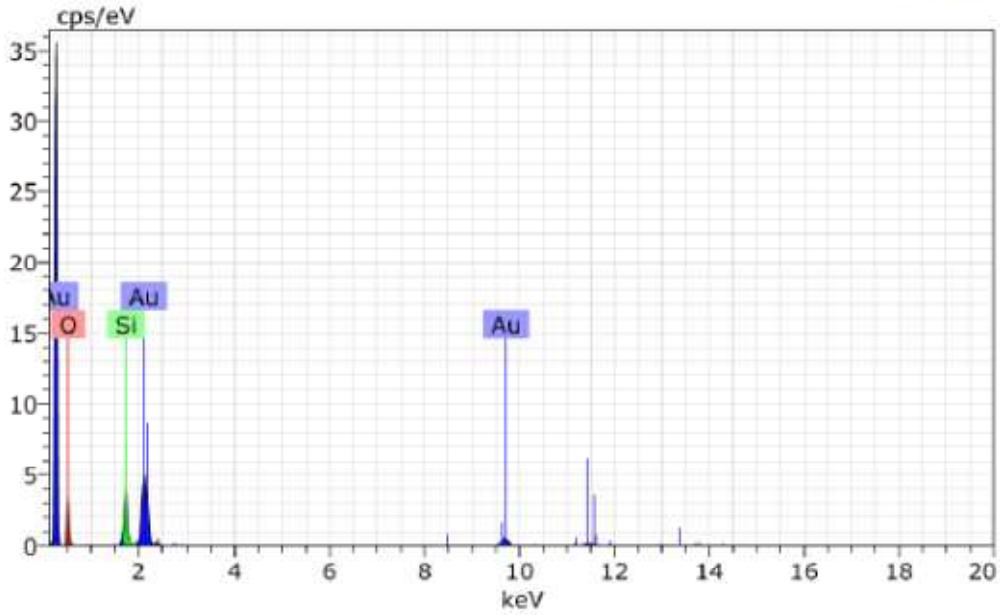


Figure 4. FESEM image of silica nanoparticles at higher magnification.



Spectrum: Sample 1 7

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
O	8	K-series	20243	3.60	67.92	78.80	0.49
Si	14	K-series	33836	1.70	32.08	21.20	0.10
Au	79	L-series	14318	0.00	0.00	0.00	0.00
		Total:		5.30	100.00	100.00	

Figure 5. EDS result of silica nanoparticles obtained in FESEM study.

The purpose for using CTAB molecules as template is to generate pores inside the silica nanoparticles. In order to establish that the synthesized silica nanoparticle is indeed formed through CTAB template, we conducted a control experiment. In absence of any CTAB, we added just the tetraethyl orthosilicate (TEOS) in basic medium but no precipitate of silica appeared. In this condition, CTAB was added next and a white precipitate was obtained immediately after the addition of surfactant. The resulting

material was washed, dried and analyzed by FESEM. The obtained micrograph shows that instead of 100 nm sized silica nanoparticles, there is a formation of silica agglomerates with no well-defined discrete particle morphology (Figure 6). The result of this control experiment indicated that CTAB molecules when added prior to the addition of TEOS, generated silica nanoparticles with an average diameter of 100 nm. Since the role of the CTAB molecules is to generate pores in silica nanoparticles, we indirectly arrive at a conclusion that these silica nanoparticles are expected to contain pores within its structures. The pore structures of these silica nanoparticles and the surface area of these materials has been characterized by N_2 gas adsorption study.

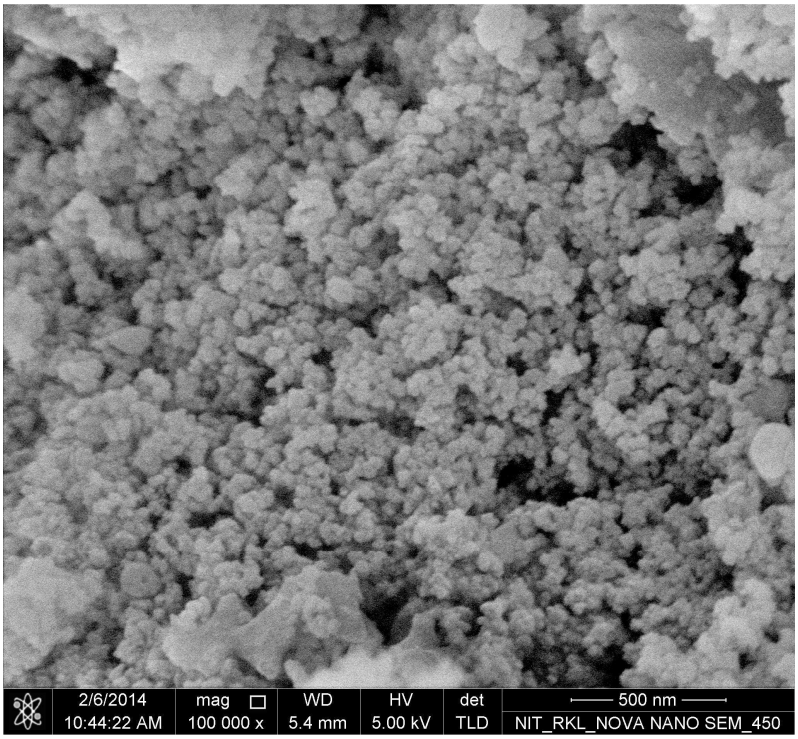


Figure 6. FESEM image of the material generated by control experiment

Further characterization of silica nanoparticles were performed using FTIR spectroscopy. The FTIR spectrum of surfactant unwashed silica nanoparticles is shown in Figure 7. The characteristic CTAB peaks are evident at 2919 cm^{-1}, 2890 cm^{-1} and at 1484 cm^{-1}, respectively [8].

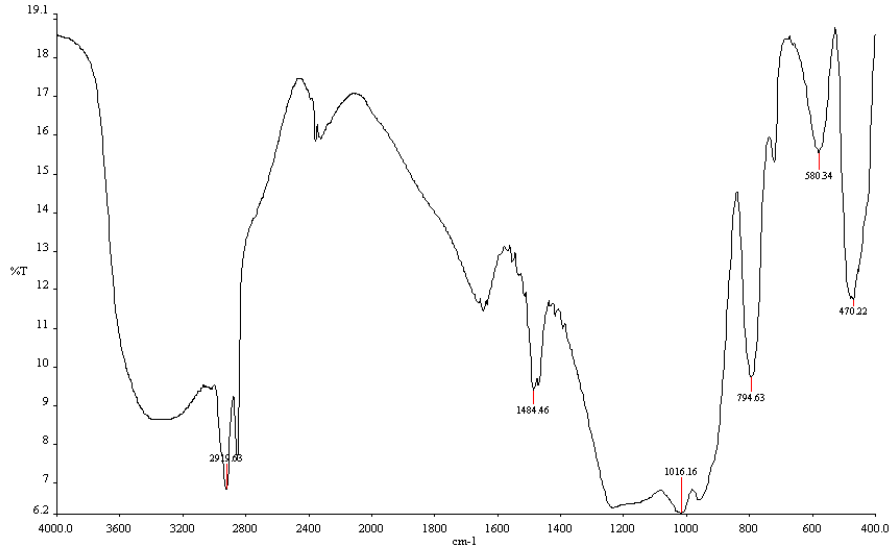


Figure 7. FTIR spectrum of unwashed silica nanoparticles

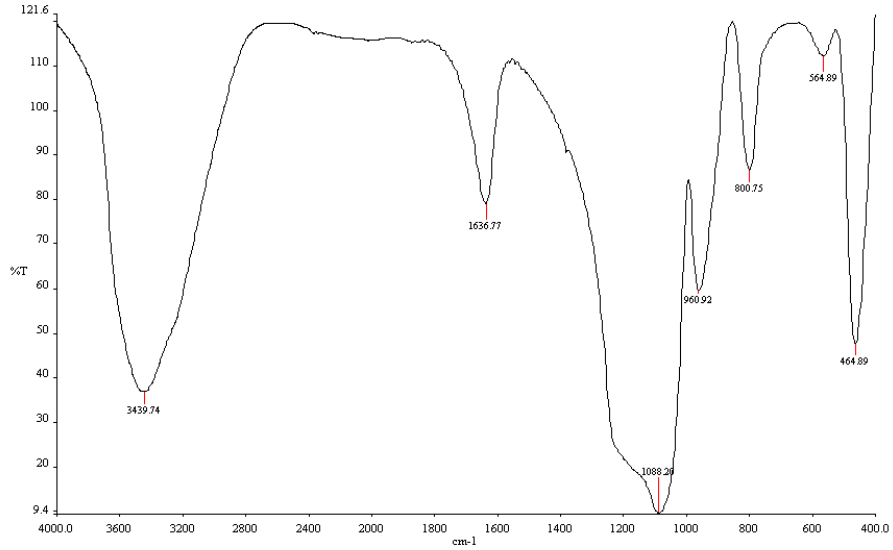


Figure 8. FTIR spectrum of surfactant washed silica nanoparticles

The broad vibration peaks at 3400 cm^{-1} and 1050 cm^{-1} confirms the silanol O-H stretching and Si-O stretching, respectively. In the FTIR spectrum of surfactant washed sample, only the characteristic peaks of CTAB is absent as expected and confirms the successful removal of the surfactant molecules.

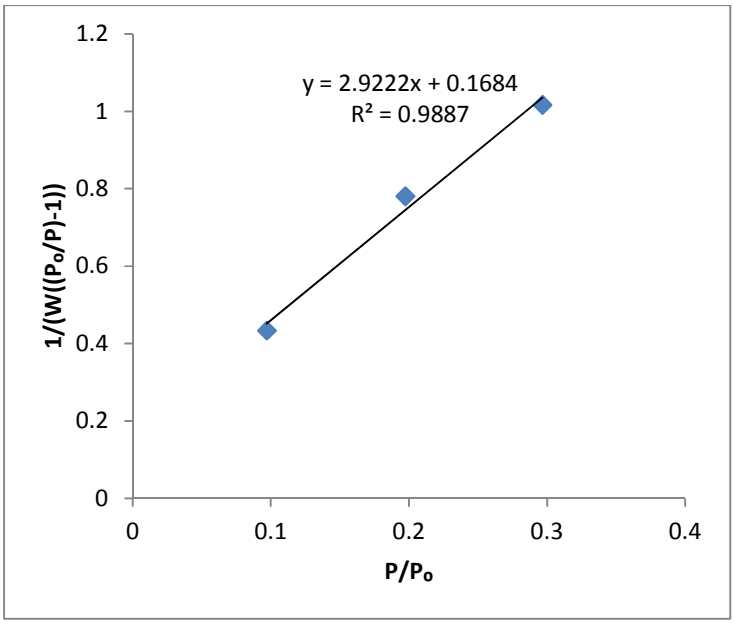


Figure 9. Plot of N_2 gas adsorption data according to BET equation of linear form

We have carried out N_2 gas adsorption analysis of surfactant washed silica nanoparticles. The results have been plotted to fit the BET linear equation as shown in Figure 9. From the slope and the intercept, we obtained the value of monolayer volume, which yielded the specific surface area of 1127 m^2/g of silica nanoparticles upon calculation. This result indicates that the silica nanoparticles prepared by this surfactant template method are indeed mesoporous in nature. Such high surface area can only be accounted for if there is the presence of porous channels in mesopore dimension. A further study of the porous structure is being carried out at present.

Part B: characterization of functionalized silica nanoparticles prepared by co-condensation method

We extended our strategy of synthesizing surfactant template guided silica materials to prepare organic group functionalized silica nanoparticles. We synthesized a primary amine functionalized as well as a thiol functionalized silica nanoparticles by a process known as co-condensation method. To prepare primary amine functionalized silica, 3-aminoproyltriethoxysilane (3-APTES) was added along with TEOS during the synthesis.

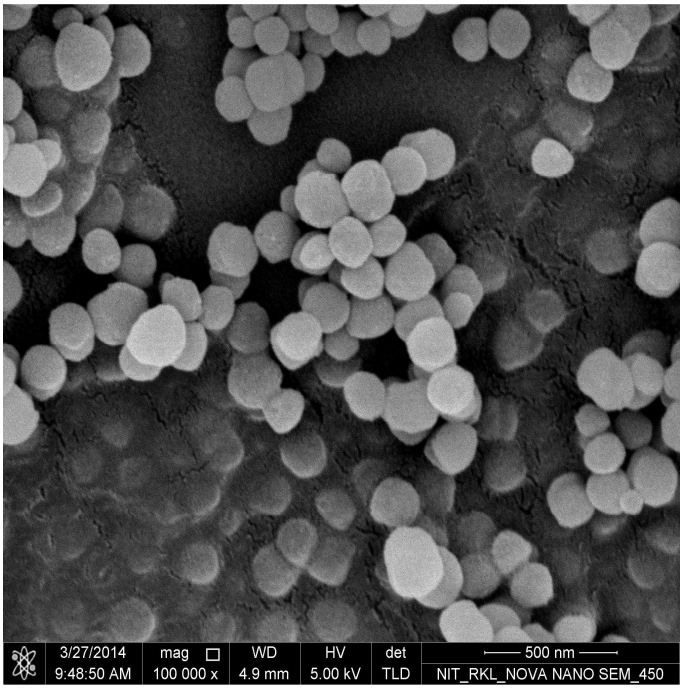


Figure 10. FESEM image of amine functionalized silica nanoparticles

The aminopropyl group is expected to be incorporated in the pore walls of silica nanoparticles. Upon removal of the surfactant template, morphology of such amine functionalized silica nanoparticles were found to be spherical with an average diameter of 100 nm as shown in the FESEM image (Figure 10). For synthesis of thiol

functionalized silica nanoparticles, 3-mercatpropyltriethoxysilane was added along with TEOS during the condensation step, propyl-mercaptan group is expected to be incorporated in the structure of silica nanoparticles. The obtained silica nanoparticles were found to have an interesting rod like morphology after removal of surfactants (Figure 11). The nanoparticles have average length of 200 nm and average width of 90 nm. Both amine and thiol functionalized silica nanoparticles were further characterized by FTIR spectroscopy (Figure 12-13).

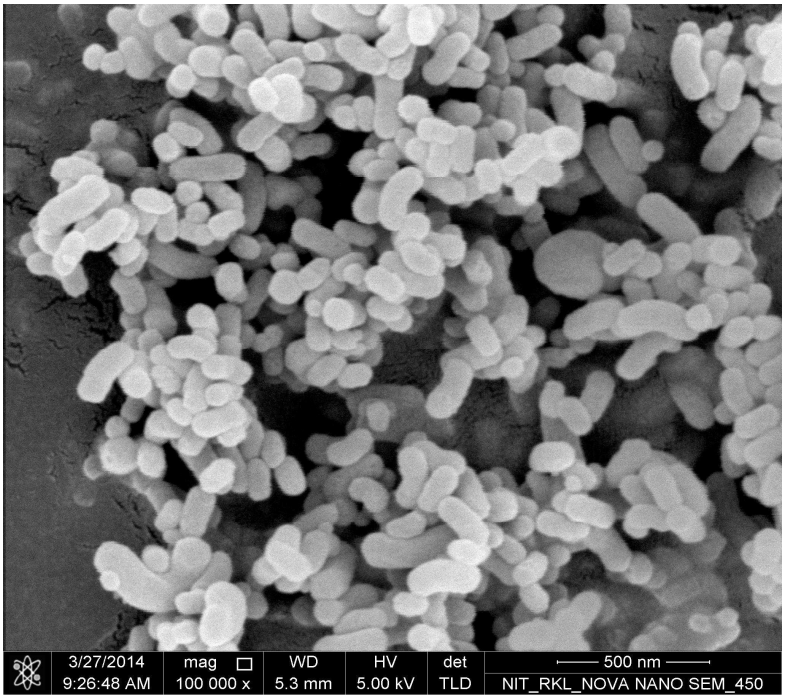


Figure 11. FESEM image of thiol functionalized silica nanoparticles

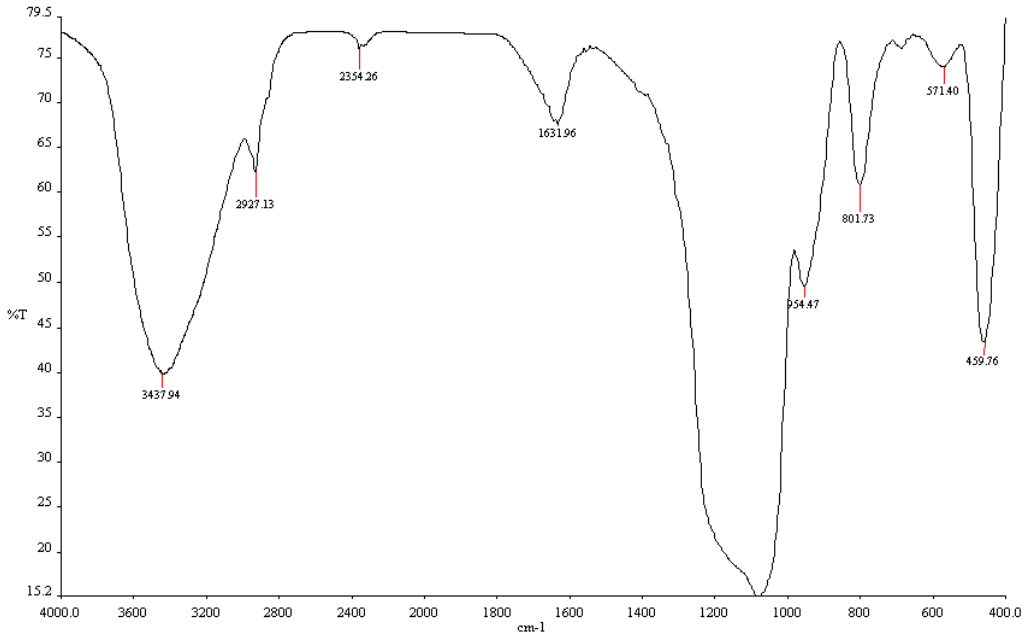


Figure 12. FTIR spectrum of primary amine functionalized silica nanoparticles

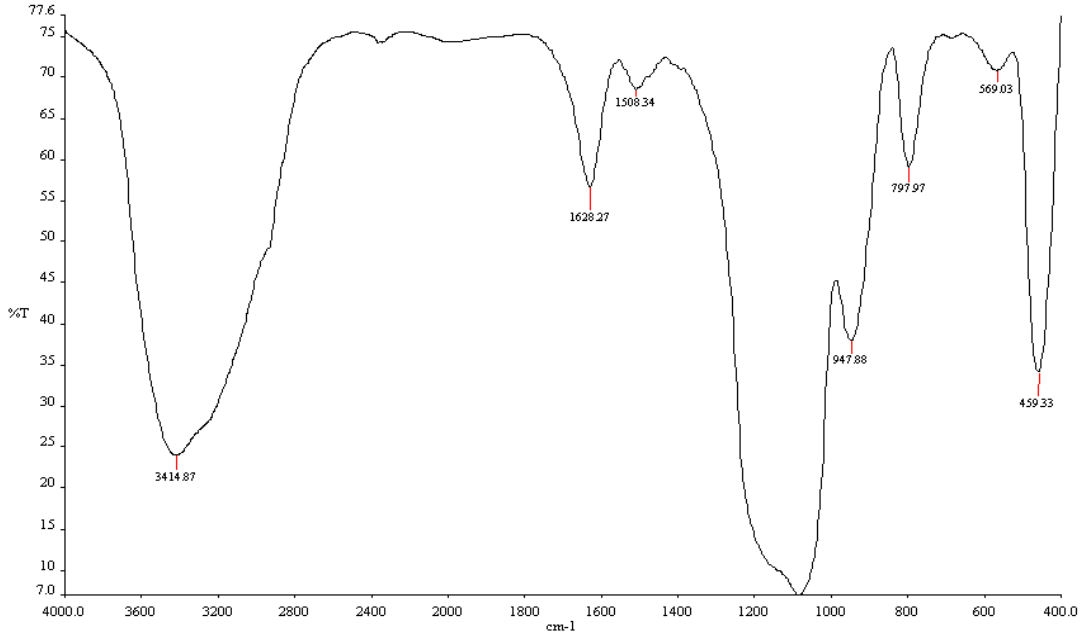


Figure 13. FTIR spectrum of thiol functionalized silica nanoparticles

Part C: study of drug loading using silica nanopartilces and guar gum capping

We selected 5-flurouracil (5-FU) drug molecules for loading inside silica nanoparticles. We chose the anticancer drug 5-FU because it is water soluble and small enough in size to diffuse inside the porous channels of silica nanoparticles. The loading and subsequent release of the drug molecules can be tracked by uv-visible spectroscopy as 5-FU shows a prominent absorption maxima (λ_{max}) at around 266 nm.

In the first step, absorption values of aqueous solutions of 5-FU at 266 nm were recorded against different concentrations and the results were plotted in the form of a standard graph (Figure 14). In order to demonstrate that the silica nanoparticles do not show significant loading of drug molecules in absence of any capping agents, we conducted a control experiment. We incubated surfactant washed silica nanoparticles (unfunctionalized) in concentrated 5-FU solution in pH = 7.4 and found almost no change of concentration of the drug solution before and after the drug loading experiment (Table 1). This result indicates that without any capping agent, the open ended channels of silica nanoparticles are not able to retain any appreciable concentration of drug molecules.

Next, we attempted to coat the silica nanoparticles with guar gum polymer in absence of any drug solution to establish the feasibility of guar cum capping. In order to find out the most suitable pH of the solution in which guar gum would make efficient bonding with silica nanoparticles, we conducted the capping experiment in pH = 1.2, pH = 7.4 and pH = 9.6, respectively.

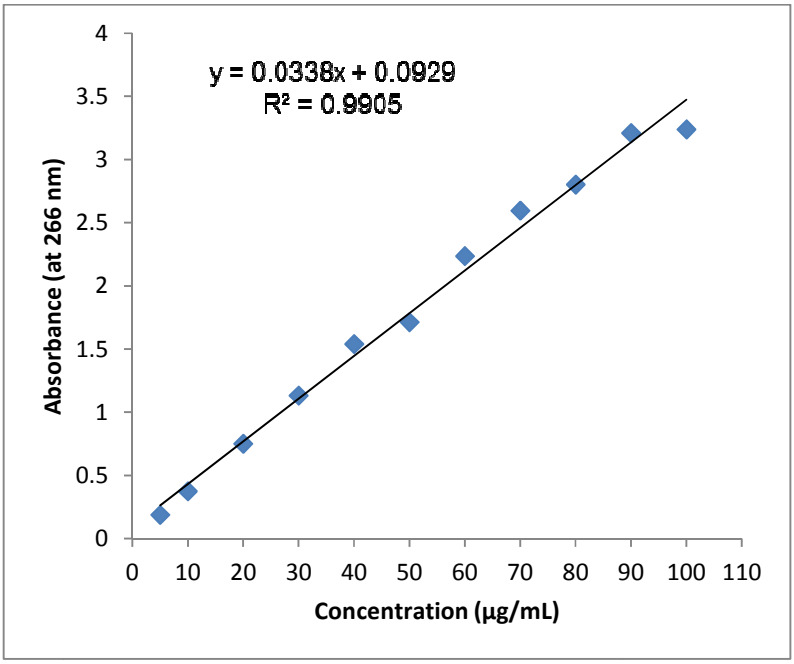


Figure 14. Standard plot of absorbance *vs.* concentration in uv-visible study for 5-FU

TABLE 1.

DRUG LOADING EXPERIMENT

Silica nanoparticles loading of 5-FU of (Uncapped):

(Absorbance data recorded at 266nm)

Concentration of drug solution before loading	Concentration of drug solution after loading
100 µg/ mL	99.81 µg/ mL

Silica nanoparticles loading of 5-FU of conc. 80 µg/mL(Capped with 0.1% Guar gum):

(Absorbance data recorded at 266nm)

Concentration of drug solution before loading	Concentration of drug solution after loading
80 µg/mL	56.48 µg/mL

The dried samples of guar gum capped silica were analyzed by FTIR spectroscopy to find the association between the carbohydrate polymer and silica nanoparticles. FTIR spectra of guar gum capped silica nanoparticles carried out in pH 1.2, 7.4 and 9.6 are represented in Figure 16, 17 and 18, respectively; while that of pure guar gum polymer is shown in Figure 15. Analysis of these FTIR spectra confirms the presence of guar gum layer on silica nanoparticles in each one of these silica samples.

Finally, we used 5-FU solution in PBS buffer (pH = 7.4) to load the drug molecules in silica nanoparticles followed by the in situ addition of 0.1% (by volume) guar gum for capping process. At the end of the capping procedure, we found an appreciable difference of absorbance values of 5-FU solution before and after the drug loading experiment. Upon calculation, the loading was found out to be 2.3×10^{-8} moles of 5FU drug per mg of silica nanoparticles. This result supports our hypothesis of trapping of anticancer drugs inside the porous channels of silica nanoparticles.

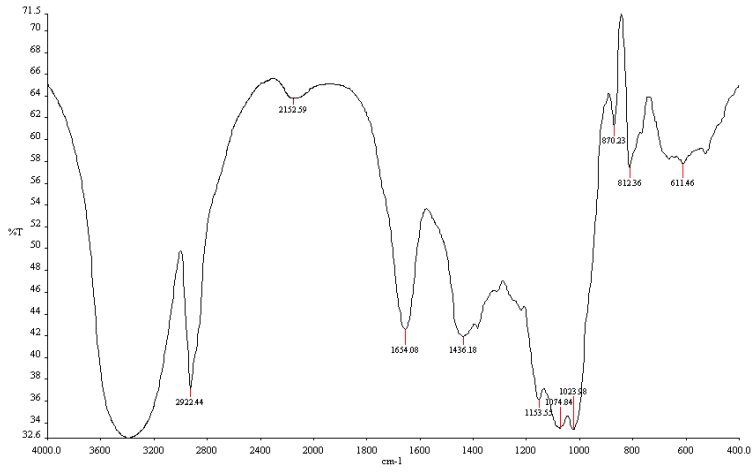


Figure 15. FTIR characterization of Guar gum polymer

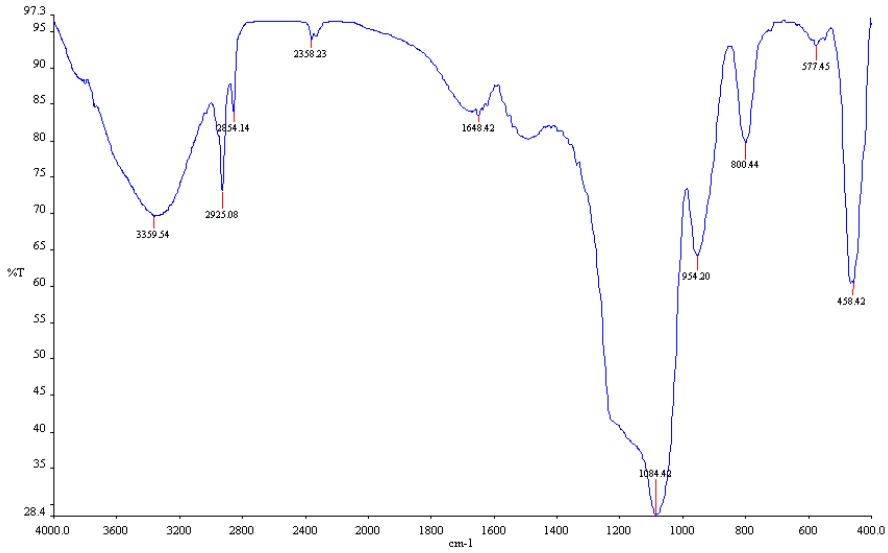


Figure 16. FTIR characterization of guar gum coated silica nanoparticles from guar gum solution of pH = 1.2

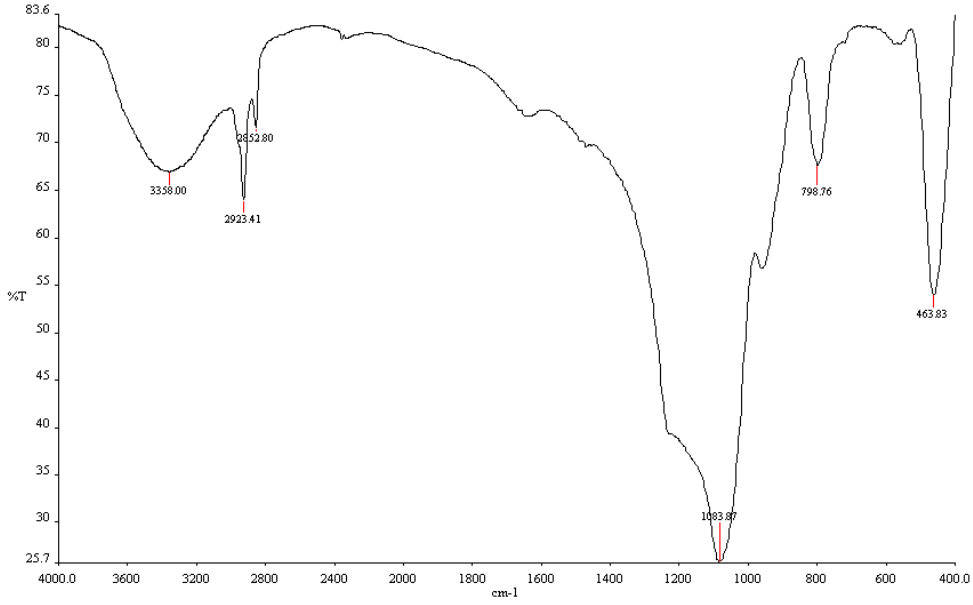


Figure 17. FTIR characterization of guar gum coated silica nanoparticles from guar gum solution of pH = 7.4

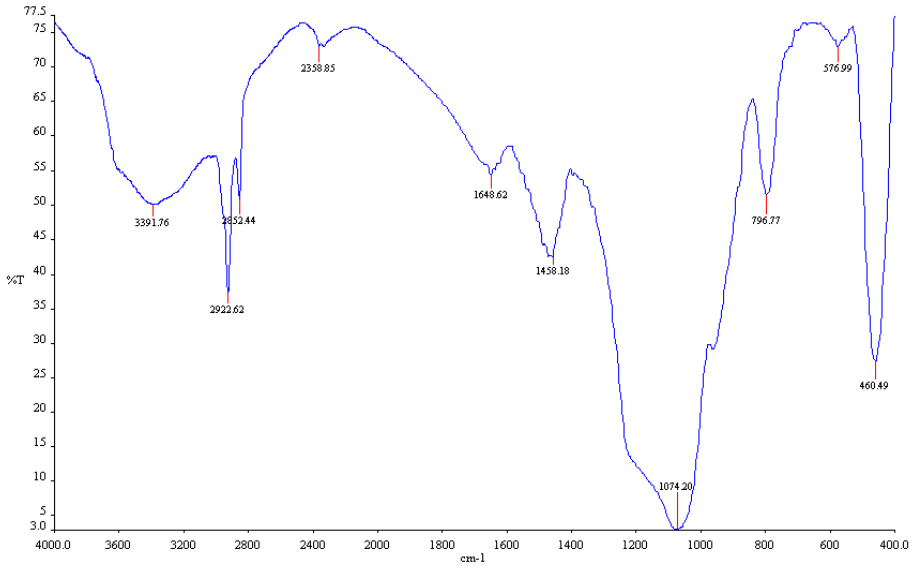


Figure 18. FTIR characterization of guar gum coated silica nanoparticles from guar gum solution of pH = 9.6

CONCLUSION

In this project we aimed to develop a silica nanoparticle based drug delivery system, which is designed to be applicable in colon cancer therapy. We have synthesized template guided silica nanoparticles of average 100 nm sizes. The involvement of surfactant template in the synthesis method indirectly indicates the presence of porous channels inside the particles, which is supported by the measured surface area of 1127 m^2/g by BET analysis. We have utilized a carbohydrate based polymer called guar gum as capping agents to trap anticancer drug molecules inside the silica nanoparticles. Also, we have successfully demonstrated an appreciable loading of the cancer drug inside the capped nanoparticles. In a separate control experiment we have also established that in absence of guar gum coating, it is not possible to retain any drug molecules inside the silica nanoparticles.

In future, we plan to demonstrate the in vitro release of the anticancer drug from the guar gum capped silica nanoparticles by adding β-D-glucosidase enzyme as release trigger. Successful demonstration of this experiment will confirm the proof-of-principle application of guar gum capped silica nanoparticles as drug delivery agents for colon cancer therapy. Our long term plan is to apply this drug delivery system in small animals for *in vivo* demonstration of our strategy. We believe that our silica nanoparticle based delivery system has great potential to be applied as oral drugs for colon cancer therapy.

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